Org Divers Evol
DOI 10.1007/s13127-016-0294-2

ORIGINAL ARTICLE

ORGANISMS DIVERSITY & EVOLUTION

From a lost world: an integrative phylogenetic analysis of *Ansonia* Stoliczka, 1870 (Lissamphibia: Anura: Bufonidae), with the description of a new species

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Received: 18 December 2015 / Accepted: 30 June 2016

Abstract While the island of Borneo is considered a global biodiversity hotspot, the species richness in many groups remains unknown and appears underestimated. During herpetological surveys carried out in the interior of Sarawak, East Malaysia, several individuals of a small species of the genus *Ansonia* Stoliczka 1870 were collected on the Usun Apau plateau and in the Gunung Hose mountain range (*Ansonia* sp. Usun Apau). An integrative taxonomic approach comprising phylogenetic (2.4 kb mitochondrial rDNA fragment, Bayesian Inference and Maximum Likelihood, >5.1 % to its closest relative) and morphometric analyses (25 measurements, multivariate ratio analysis and linear discriminant analysis), as well as morphological comparisons support the status of this operational taxonomic unit as a separate taxon at species level. The obtained phylogenetic hypothesis corroborates the two major clades within *Ansonia* found in previous studies. Within Clade One *Ansonia* sp. Usun Apau and the enigmatic *Ansonia torrentis* are part of a monophyletic group of the Bornean species *Ansonia hanitschi*, *Ansonia minuta*, *Ansonia platysoma*, *Ansonia spinulifer*, *Ansonia vidua*, and two additional undescribed taxa. This subclade must be considered as the result of an on-island radiation in the complex evolution of *Ansonia*. The new species is formally described including the identification of diagnostic morphometric traits. *Ansonia* sp. Usun Apau is endemic to two isolated mountain ridges in central Sarawak and must be considered as a new element of the unique diversity of the Bornean amphibian fauna that is potentially threatened by habitat loss at least in parts of its range.

Keywords *Ansonia teneritas* · New species · Integrative taxonomy · Phylogeny · Diversity · Evolution · Morphometrics · Usun Apau · Borneo · Mitochondrial DNA

Electronic supplementary material The online version of this article (doi:10.1007/s13127-016-0294-2) contains supplementary material, which is available to authorized users.

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Introduction

The Usun Apau plateau is one of the last inaccessible and uninhabited areas of Sarawak, Borneo, in East Malaysia. A volcanic plateau located at the headwaters of the Baram and Rajang rivers near the Indonesian border, it is protected today as a national park but was unknown to western science until 1951, when Tom Harrisson, the curator of the Sarawak Museum in Kuching, visited the site (Arnold 1957). During our herpetological expedition to the Usun Apau National Park in 2010, a series of individuals of a small species of the genus *Ansonia* Stoliczka 1870 was collected (Fig. 1). These were initially regarded during field work as conspecific with either *Ansonia hanitschi*, *Ansonia minuta*, or *Ansonia platysoma* due to their small size and overall similarity in external morphology. Subsequent

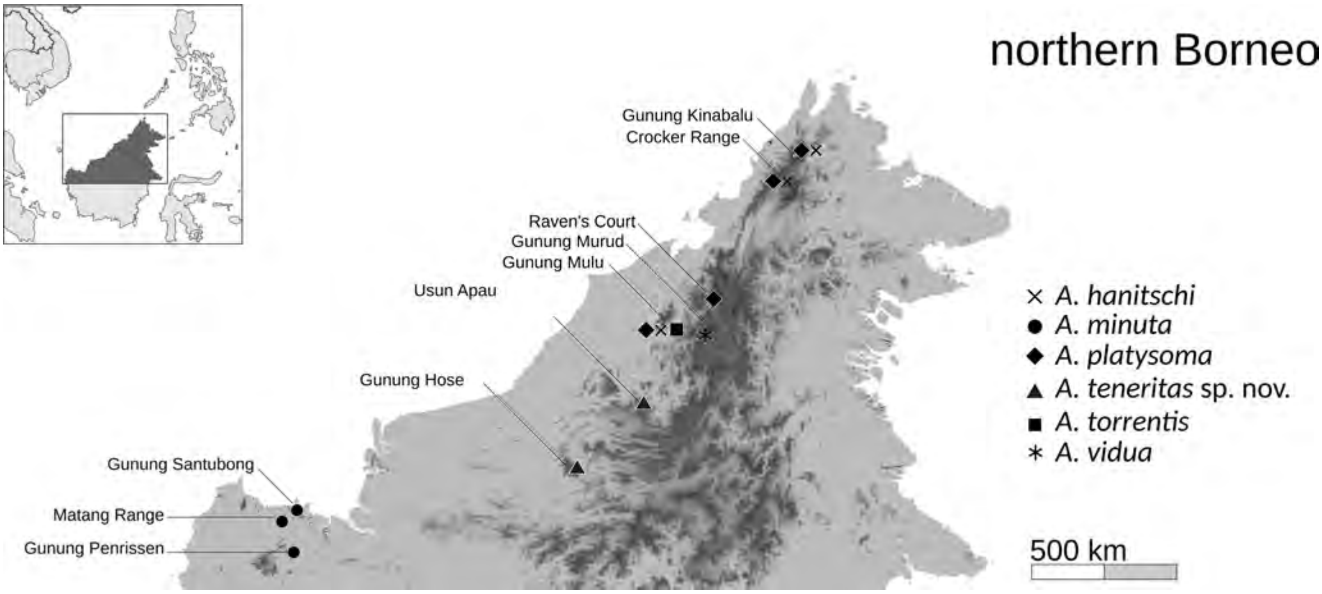

Fig. 1 Known records of *Ansonia* sp. Usun Apau and related species

examinations of the collected material, including genetic analyses (see below), however, cast doubt on this initial assignment and on the taxonomic status of this new operational taxonomic unit (OTU). A single specimen collected in 2011 in the Gunung Hose National Park, Sarawak, East Malaysia, was, on the basis of genetic evidence, also assigned to this OTU (hereinafter termed *Ansonia* sp. Usun Apau).

The 28 species of *Ansonia*, known as slender or stream toads, currently described are distributed primarily on Sundaland but also on mainland South-east Asia and the Philippines (Frost 2015). Species of this genus are characterized as adults by the combination of a slender body, indistinct subarticular tubercles, long and slender legs (relative to other bufonids), membranous digital webbing, the absence of parotid glands, and a visible tympanum. Additionally, the larval forms exhibit oral suckers as an adaptation for life in streams or rivers with a moderate to strong current (Inger 1960, 1966), except for *Ansonia leptopus* (Inger 1992). According to current knowledge of the phylogenetic relationships within *Ansonia*, the Bornean species do not form a monophyletic group but are divided over two major clades (Hertwig et al. 2014; Matsui et al. 2010). Clade One consists of species from the Malay Peninsula, Thailand, and the following species from Borneo: *Ansonia hanitschi*, *Ansonia spinulifer*, *Ansonia platysoma*, *Ansonia minuta*, and *Ansonia vidua*, and one undescribed species not identical with *Ansonia* sp. Usun Apau (Hertwig et al. 2014; Matsui et al. 2010). Clade Two comprises species from the Malay Peninsula, the Philippines, and the Bornean taxa *Ansonia albomaculata*, *Ansonia longidigita*, *Ansonia leptopus*, *Ansonia fuliginea*, *Ansonia guibei*, as well as one undescribed species (Hertwig et al. 2014; Matsui et al. 2010). In the study by Matsui et al. (2010), *Ansonia torrentis* was also assigned to the Clade Two but its position was later questioned by Hertwig et al. (2014). The real diversity of *Ansonia* is still unclear, as demonstrated by the fact that several new species have been described in recent years. *Ansonia lumut* from Terengganu, West Malaysia, and *Ansonia vidua* from Sarawak, East Malaysia, for example, were only described in 2014 (Chan et al. 2014; Hertwig et al. 2014). Matsui et al. (2010) identified five additional OTUs using molecular data, but these remain unnamed.

This study examines the phylogenetic relationships and taxonomic status of *Ansonia* sp. Usun Apau using morphological, morphometric, and genetic analyses. The phylogenetic hypothesis obtained is based on a larger sample of *Ansonia* than that featured in previous studies and is used to discuss the evolutionary and biogeographic relationships between this OTU and related taxa. As the result of the investigation, we provide a formal description of *Ansonia* sp. Usun Apau as a new species of *Ansonia*. In addition, we discuss the contribution of morphometric measurements to the diagnosis and determination of cryptic species (according to the definition of Bickford et al. 2006) within this genus.

Material and methods

Sampling and markers

Specimens and tissue samples of *Ansonia* from Borneo, now held in the collection of the Natural History Museum Bern (NMBE), the Zoological Museum Hamburg (ZMH), and the Institute of Biodiversity and Environmental Conservation (IBEC, UNIMAS), were collected between the years 2009 and 2014 during several field trips to Sabah and Sarawak with the kind permission of the Sabah Parks and the Sarawak Forest

Department (NPW.907.4.2(IV)-3, NPW.907.4.4(V)-63, NPW.907.4.4(V)-99, NCCD.907.4.4(Jld.VI)-106, NCCD.907.4.4(Jld.9)-19; park permits 3/2009, 23/2010, 53/2010, 56/2011, 62/2011, 038/2012, 035/2013; export permits 08169, 08961, 09813, 10644, 14603). Specimens were anesthetized and euthanized in ca. 2 % aqueous Chlorobutanol solution (1,1,1-trichloro-2-methyl-2-propanol). Tissue samples were taken from either liver or femoral muscles in adult toads or from the tail musculature in tadpoles and stored in RNALater buffer solution (Ambion/Applied Biosystems). Specimens were then preserved in 4 % neutral buffered formalin for at least 2 weeks, washed in tap water, and transferred to 75 % ethanol in incremental steps of 30 and 50 % to avoid shrinkage.

We examined DNA sequences of the mitochondrial 12S and 16S rRNA genes and the intervening tRNA valine from 40 specimens (Table 1, Appendix), in combination with 79 sequences from GenBank (Hertwig et al. 2014; Matsui et al. 2010). The outgroup was selected following Matsui et al. (2010) and Pyrons and Wiens (2011) and comprised *Pedostibes maculatus* (*Sabahphrynus maculatus*, Chan et al. 2016), *Pedostibes hosii* (*Rentapia hosii*, Chan et al. 2016), *Leptophryne borbonica*, *Duttaphrynus melanostictus* and *Pelophryne brevipes* (Appendix).

Laboratory protocols

Total genomic DNA was extracted from liver tissue or macerated muscle using Wizard SV Genomic DNA Purification System (Promega, Switzerland). Eight primer combinations were used (Table 1). For PCR reactions, a 25-μl PCR reaction volume containing 2 μl of DNA, 12.5 μl of GoTaq Hot Start Green Master Mix (Promega), 2 μl of each primer (10 μM), (for 16S rDNA 1 μl [20 μM]), and 6.5 μl ddH$_2$O (for 16S rDNA 8.5 μl ddH$_2$O) was used. Cycling conditions for 12S rRNA and tRNA-Val were denaturation at 94 °C for 2 min; 35 cycles at 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 h and 10 min and then one final extension cycle at 72 °C for 7 min. The cycling conditions for 16S rRNA were as follows: denaturation at 94 °C for 2 min, 35 cycles at 94 °C for 30 s, 48.2 °C for 30 s, and 72 °C for 1 min, followed by one final extension at 72 °C for 5 min. PCR products from 16S rDNA were cleaned using Wizard® SV Gel and PCR Clean-Up System (Promega). 12s and tRNA-Val PCR products were purified and sequenced by Microsynth (Microsynth GmbH, Balgach, Switzerland). Sequences were checked manually for ambiguities in Geneious Pro 7.0.6 (Kearse et al. 2012) and assigned standard IUB codes.

Phylogenetic analyses

The markers were aligned using the MAFFT plug-in in Geneious Pro (Katoh and Standley 2013). After each marker had been checked for contamination and sequencing and laboratory errors separately, the sequences were concatenated. Uncorrected genetic p-distances were calculated in Geneious Pro. The best-fitting models of sequence evolution for subsequent analyses were chosen for each gene partition using PartitionFinder v1.0.1 (Lanfear et al. 2012) with linked branch lengths.

Bayesian inference (BI) was carried out in MrBayes 3.2 (Huelsenbeck and Ronquist 2001). Two independent runs of Metropolis-coupled Markov chain Monte Carlo analyses were conducted, each consisting of three heated and one cold chain with a default temperature of 0.2. The analyses were run for 50 million generations with a sampling frequency of 100. The first 25 % of samples were discarded as burn-in (12,500,000 trees) once trace files had been checked with Tracer v.1.5 (Rambaut et al. 2014) to assess convergence between the two independent runs.

Maximum likelihood (ML) analysis was performed in RAxML V8 (Stamatakis 2014) using the GTR GAMMA model of nucleotide substitution. Internal node support was verified with 300 bootstrap replicates. The appropriate bootstrapping number was estimated using bootstopping criteria as recommended by Pattengale et al. (2009).

Clades were considered to be robustly supported when clade credibility values were ≥0.95 in BI (Huelsenbeck and Ronquist 2001) and with bootstrap values ≥70 % in ML (Hillis and Bull 1993). The number of autapomorphic substitutions was calculated in MacClade v.4.08a (Maddison and Maddison 2005).

Table 1 Primers used for the amplification of the ~2400 bp mitochondrial rDNA fragment

Marker	Primer name	Primer sequence (5′-3′)	Reference
rDNA	tRNAphe	GCRCTGAARATGCTGAGATGARCCC	Goebel et al. (1999)
	tRNAval-H	GGTGTAAGCGARAGGCTTTKGTTAAG	Goebel et al. (1999)
	12 L1	AAAAAGCTTCAAACTGGGATTAGATACCCCACT	Goebel et al. (1999)
	16SH	GCTAGACCATKATGCAAAAGGTA	Goebel et al. (1999)
	12Sm	GGCAAGTCGTAACATGGTAAG	Pauly et al. (2004)
	16Sa	ATGTTTTTGGTAAACAGGCG	Goebel et al. (1999)
	16SC	GTRGGCCTAAAAGCAGCCAC	Pauly et al. (2004)
	16SD	CTCCGGTCTGAACTCAGATCACGTAG	Pauly et al. (2004)

Morphometric analyses

For morphometric analyses, 25 traits (Fig. 2, see Table 2 for abbreviations, supplementary material) were measured in 13 individuals of the *Ansonia* sp. Usun Apau including the single specimen from Gunung Hose, 17 *Ansonia minuta*, 13 *Ansonia platysoma*, 5 *Ansonia hanitschi*, and 3 *Ansonia torrentis* (supplementary material). These taxa were selected on the basis of their close phylogenetic relationships with and similarity in external morphology to *Ansonia* sp. Usun Apau. *Ansonia vidua* was not considered due to its significantly larger body size and divergent morphology (Hertwig et al. 2014). The sex of the specimens was determined on the basis of differences in snout-vent length (SVL) (presence or absence of nuptial pads, vocal sacs, and ova visible through skin at the inguinal region). Specimens that could not be sexed with certainty on the basis of external traits were dissected and determined by inspecting the primary sexual organs.

Morphometric traits and corresponding abbreviations (Table 2 and Fig. 2) conform to those used in previous studies (e.g., Boulenger 1880, 1897; Gascon et al. 1996; Heyer 1984; Matsui 1984; Okada 1931). Measurements were taken from formalin- and ethanol-preserved specimens using a calibrated Leica DFC420 camera on a Leica MZ16 with motorfocus and measured from digital images in ImageJ (Schneider et al. 2012). Two characters (HDP and HDA) were measured using a digital caliper. The measurements were taken from the side of the specimen that was in better shape in terms of quality of preservation.

We carried out the multivariate ratio analysis (MRA) put forward by Baur and Leuenberger (2011), which permits a strict separation of differences in size and shape and the interpretation of principal components as ratios (cf. Bauer and Leuenberger 2011). We used the geometric mean of the original measurements to define an isometric size axis (“isosize”). This resulting isosize axis comprises differences in scaling of size only. We then obtained size-independent shape variables by projecting the measurements orthogonal to isosize. This allowed us to calculate a principal component analysis (PCA) accounting exclusively for differences in proportions in the covariance matrix of the shape parameters. Matrix scatterplots were produced to show error variance. After a preliminary analysis, body width (BW) and axilla to groin distance (AGD) were omitted from the dataset due to an obviously high level of measurement errors caused by preservation artifacts. Separate analyses of the sexes led to an improved separation of the OTUs. Only for the linear discriminant analysis (LDA) ratio extractor (Baur and Leuenberger 2010) were the sexes combined to obtain a larger sample size for each species. The LDA ratio extractor was used to find those body ratios that permitted pairwise differentiation between the species. R language for statistical computing was used for data analysis (R Studio, integrated development environment for R; version 0.98.1073).

Following comparative material was examined (institutional abbreviations are based on Sabaj Pérez (2012), except following local usage, we retain ZRC for the Lee Kong Chian Museum of Natural History, National University of Singapore, the abbreviation used in Sabaj Pérez 2012 is USDZ):

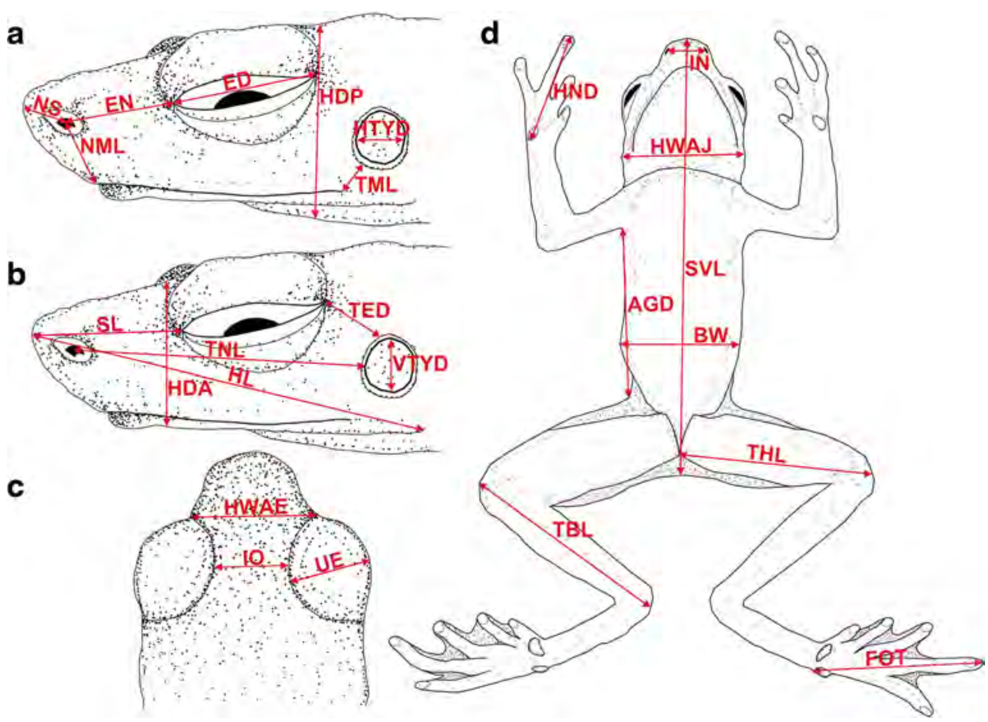

Fig. 2 Morphometric measurements used in this study (see Table 2)

Table 2 Abbreviations of morphometric measurements used in this study (see Fig. 1)

Abbreviation	Character name	Definition
SVL	Snout-vent length	From tip of snout to vent
BW	Body width	Greatest width of body
AGD	Axilla to groin distance	Distance between posterior edge of fore limb insertion and anterior edge of hind limb insertion
HL	Head length	Distance between angle of jaws and tip of snout
HWAE	Head width at the anterior of the eye	Width of the head taken anterior of the eye
HWAJ	Head width at the angle of jaws	Distance between angle of jaws
SL	Snout length	Distance from anterior margin of eye to tip of snout
NS	Nostril-snout distance	Distance between centre of nostril and tip of snout
EN	Eye-nostril distance	Distance between anteriormost point of eye and centre of nostril
TNL	Tympanum-nostril length	Distance from anterior margin to centre of nostril
TML	Tympanum-mouth length	Shortest distance between anterior rim of the tympanum to mouth (maxilla)
NML	Nostril-mouth length	Shortest distance between the centre of the nostril to mouth (maxilla)
IN	Internarial distance	Distance between outer rims of nostrils
ED	Eye diameter	Horizontal diameter of exposed portion of eyeball
UE	Upper eyelid width	Greatest transverse width of upper eyelid
IO	Interorbital distance	Smallest distance between upper eyelids
TED	Tympanum-eye distance	Shortest distance between anterior rim of tympanum and posterior edge of eye
HTYD	Horizontal tympanum diameter	Maximum horizontal tympanum diameter, measured from the outside edges of tympanic annulus
VTYD	Vertical tympanum diameter	Maximum vertical tympanum diameter, measured from the outside edges of tympanic annulus
TBL	Tibia length	Distance between anterior surface of knee and posterior surface of heel with both tibia and tarsus flexed
THL	Thigh length	From cloaca to most distal apex of knee
HND	Hand length	Distance from base of palmar tubercle to tip of third finger
FOT	Foot length	Distance from base of inner metatarsal tubercle to tip of fourth toe
HDP	Head depth posterior of the eye	Greatest depth of head, taken posterior of eye
HDA	Head depth anterior of the eye	Greatest depth of head, taken anterior of eye

Ansonia albomaculata Inger, 1960. FMNH 81975 (holotype) and SM (uncataloged), in Bottle 51 (paratype), from "1400 to 2000 ft above sea level, in the headwaters of the Baleh River, Third Division, Sarawak"; UBD 266, 309, 318, 337, 389, 396, 412, 472, 476, 481, 487, 508, 539, 617, Batu Apoi Forest Reserve, Temburong District, Brunei Darussalam.

Ansonia guibei Inger, 1966. UNIMAS 7746; 8058, 8060. Mesilau, Gunung Kinabalu Park, Sabah, Malaysia.

Ansonia hanitschi Inger, 1960. UNIMAS 8050, 8081, Liwagu Trail, Gunung Kinabalu Park, Sabah, Malaysia; UNIMAS 8055, 8470, ZRC 1.11911, Mesilau, Gunung Kinabalu Park, Sabah, Malaysia; UNIMAS 7800, Poring, Gunung Kinabalu Park, Sabah, Malaysia; NMBE 1056271–1056280, Sungei Tapin, Gunung Mulu, Sarawak, Malaysia.

Ansonia latidisca Inger, 1966. UNIMAS-OJJ 009–011, NMBE 1061497, 1061498, Gunung Penrissen, Sarawak, Malaysia.

Ansonia leptopus (Günther, 1872). UBD 289, 382, 293, 397, 420, 506, 510, 517, 618–19, Batu Apoi Forest Reserve, Temburong District, Brunei Darussalam; NMBE 1056582, 1056591, 1057156, 1057168, 1057169, 1057172, 1059721–1059723, UNIMAS 8393, 8402, Kubah National Park, Sarawak, Malaysia; NMBE 1056281–1056283, Camp 5, Gunung Mulu National Park, Sarawak, Malaysia; UNIMAS 8725, Gunung Santubong, Sarawak, Malaysia.

Ansonia longidigita Inger, 1960. BMNH 99.8.19.12 (holotype), "…4200 ft on Mount Kina Balu, North Borneo"; UNIMAS 7925–26. Gunung Santubong, Sarawak, Malaysia; UBD 90, 94–98, 135, 160, Batu Apoi Forest Reserve, Temburong District, Brunei Darussalam; NMBE 1056284–1056288, ZMH A09368, A09371, ZRC 1.12012–13, Camp 2, Gunung Mulu National Park, Sarawak, Malaysia; ZMH A09370 8th Mile, Crocker Range Park, Sabah, Malaysia.

Ansonia minuta Inger, 1960. NMBE 1056601, 1057163, 1059725–1059728, ZMH A10003–06, ZRC 1.2215, Kubah National Park, Sarawak, Malaysia; NMBE 1057516–1057518, 1057527, 1057528, 1058244, 1061560, 1061561, Mount Penrissen, Sarawak, Malaysia; NMBE 1059717–1059719, Santubong National Park, Sarawak, Malaysia. *Ansonia platysoma* Inger, 1960. ZMH A10007-09, ZRC 1.12004, 1.12007, Camp 2, Gunung Mulu National Park, Sarawak, Malaysia; UNIMAS 7371, 7373, 7384, 7385, 7413, 7414, 7415, 7417, 7418, 7420, 7421, Crocker Range Park, Sabah, Malaysia; UNIMAS 9047, 9056, Sayap Substation, Gunung Kinabalu, Sabah, Malaysia.

Ansonia spinulifer (Mocquard, 1890). SBC A.00001, Gunung Meraja, Bau, Sarawak, Malaysia; SBC A.00032, Gunung Pambor, Bau, Sarawak, Malaysia; SBC A.00045, Gunung Ropih, Bau, Sarawak, Malaysia; SBC A.00065–67, Gunung Tai Ton, Bau, Sarawak, Malaysia; SBC A.00093, Gunung Batu Payong, Bau, Sarawak, Malaysia; SBC A.00165–66, Gunung Umbut, Bau, Sarawak, Malaysia; SBC A.00288, Gunung Podam, Bau, Sarawak, Malaysia; UNIMAS 7875, Anna Rais, base of Gunung Penrissen, Sarawak, Malaysia; UNIMAS 7020–22. Ranchan Pool, Serian, Sarawak, Malaysia; UNIMAS 7580. Gunung Gading, Sarawak, Malaysia; NMBE 1057085, 1057086, 1057171, Kubah National Park, Sarawak, Malaysia.

Ansonia torrentis Dring, 1983. NMBE 1056296–1056298, Sungei Tapin, Gunung Mulu, Sarawak, Malaysia.

Ansonia vidua Hertwig, Pui, Haas & Das, 2014. NMBE 1061645, holotype and NMBE 1066153, paratype), summit trail, Gunung Murud, Pulong Tau National Park, Sarawak, Malaysia.

Results

Phylogenetic analyses

The final concatenated alignment comprised 2465 bp, including 958 bp of 12S (1-958), 70 bp of tRNA-Val (959-1028), and 1437 bp of 16S rRNA (1029-2465). Following sequence evolution models were applied based on the test results obtained with PartitionFinder: 12S: GTR + I + G, tRNA-Val: SYM + I + G, 16S: GTR + I + G (details available upon request). Both Bayesian and ML analyses resulted in congruent consensus trees (Fig. 3) that corroborate the two major clades found in previous phylogenetic studies (Matsui et al. 2010; Hertwig et al. 2014). Within Clade One *Ansonia torrentis* and *Ansonia* sp. Usun Apau are part of a subclade consisting of the Bornean species *Ansonia hanitschi*, *Ansonia minuta*, *Ansonia platysoma*, *Ansonia spinulifer*, *Ansonia vidua*, and the undescribed taxa referred to by Matsui et al. (2010) as *Ansonia* sp. 3 and *Ansonia* sp. 4. The enigmatic species *Ansonia torrentis* is closely related to these small- to medium-sized montane *Ansonia* species from Borneo (Fig. 3).

The OTU *Ansonia* sp. Usun Apau, including the single specimen from Gunung Hose, is robustly supported as a monophyletic group (100 % bootstrapping support and 57 autapomorphic substitutions). The sister group is a clade consisting of *Ansonia platysoma*, *Ansonia vidua*, *Ansonia torrentis*, and *Ansonia* sp. 3 (Fig. 3). The genetic p-distance between the individual from Gunung Hose and the specimens from Usun Apau is 0.4–0.8 %. The genetic p-distance from this species to the valid species of *Ansonia* from Borneo is 13.4–13.8 % to *Ansonia albomaculata*, 10.1–10.9 % to *Ansonia fuliginea*, 10.4–10.7 % to *Ansonia guibei*, 6.2–6.5 % to *Ansonia hanitschi*, 11.8–12.4 % to *Ansonia leptopus*, 11.8–12.6 % to *Ansonia longidigita*, 8.3–9.5 % to *Ansonia minuta*, 6.8–7.5 % to *Ansonia platysoma*, 11.6–12.1 % to *Ansonia spinulifer*, 5.1–5.4 % to *Ansonia torrentis*, and 6.3–6.6 % to *Ansonia vidua*.

Morphometric analyses

Neither the females nor the males of *Ansonia hanitschi*, *Ansonia minuta, Ansonia platysoma*, *Ansonia torrentis*, and *Ansonia* sp. Usun Apau are separated by the combination of shape PC1 and shape PC2 (Online Resources 1 and 2), and this is particularly striking in the males. The isometric size in males and females in *Ansonia hanitschi*, *Ansonia minuta, Ansonia platysoma*, *Ansonia torrentis*, and *Ansonia* sp. Usun Apau differs gradually, with *Ansonia platysoma* and *Ansonia* sp. Usun Apau being isometrically smallest, followed by *Ansonia minuta* and *Ansonia hanitschi*. The males of *Ansonia torrentis* are isometrically largest (Online Resources 1 and 2). These results were partly influenced by allometric relationships, as shown in the ratio spectra (Online Resources 3).

The LDA ratio extractor requires an a priori assignment of individuals to an OTU. According to the LDA ratio extractor, the combination of the ratios between SVL/HD and internarial distance (IN)/nostril-mouth length (NML) separates *Ansonia* sp. Usun Apau and *Ansonia platysoma* (Fig. 4a), the combination of NML/ foot length (FOT) and thigh length (THL)/FOT separates *Ansonia* sp. Usun Apau and *Ansonia minuta* (Fig. 4b), and the combination of SVL/HD and SVL/THL separates *Ansonia minuta* and *Ansonia platysoma* (Fig. 4c). In *Ansonia hanitschi*, the number of available specimens was too low for the LDA ratio extractor.

In conclusion, we consider the apomorphic combination of genetic and external morphological characters (see description and diagnosis below) as well as the level of genetic distance and morphometric difference to be a reliable indication of the distinctiveness of *Ansonia* sp. Usun Apau as a separate taxon in the rank of a species in accordance with the phylogenetic species concept (Cracraft 1992; Nixon and Wheeler 1990), the

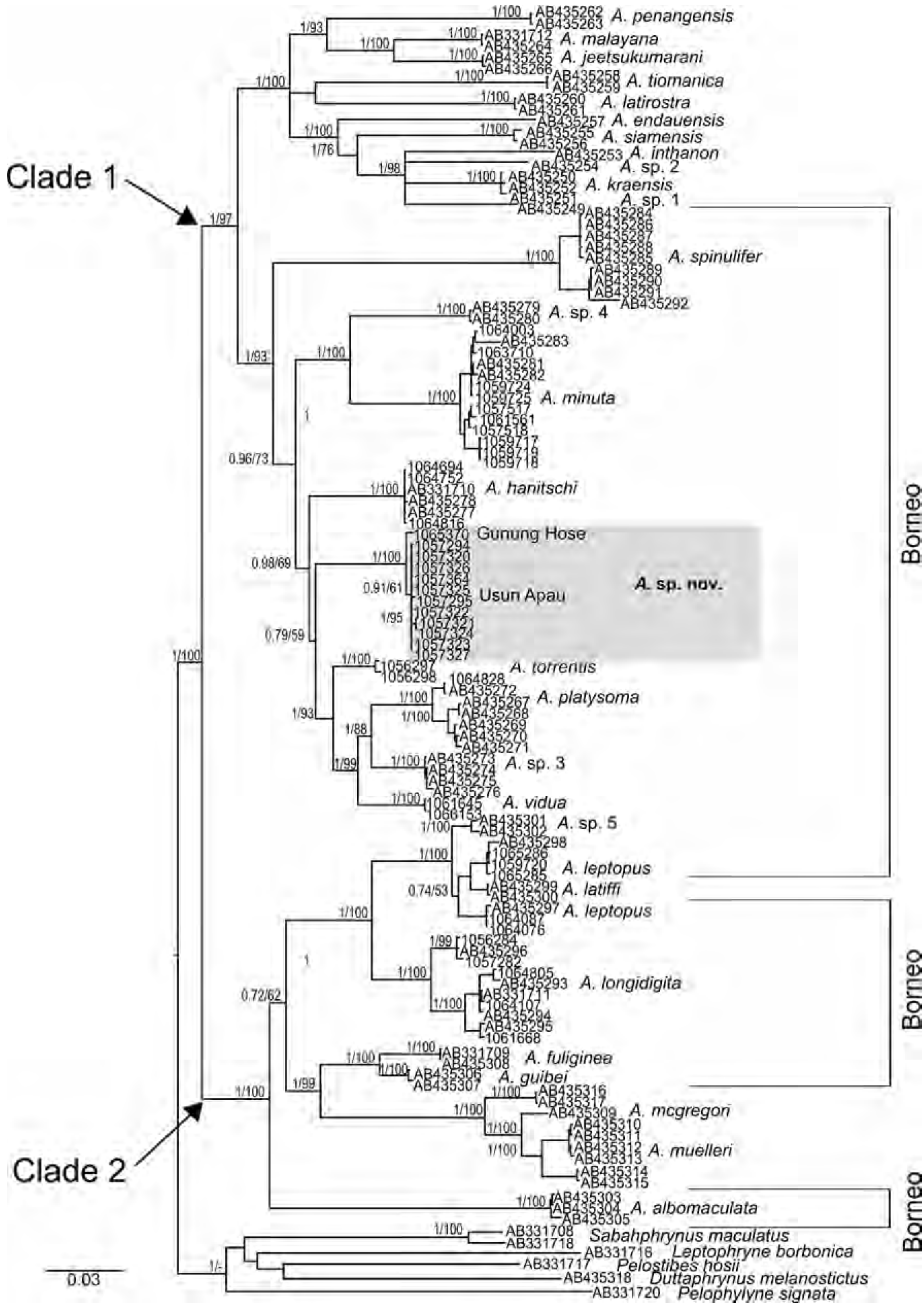

Fig. 3 Majority rule consensus tree of the BI analysis. The first value shows the Bayesian posterior probability of the node; the second value shows the ML bootstrap support

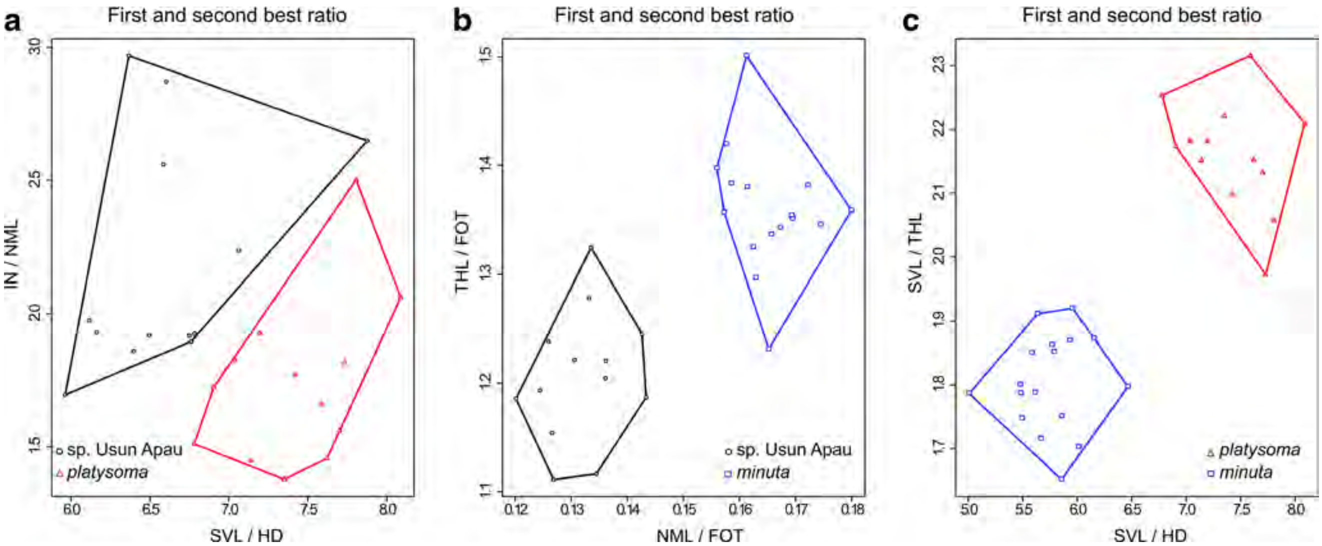

Fig. 4 First and second-best morphometric ratio for the separation of **a** *Ansonia* sp. Usun Apau and *Ansonia platysoma*, **b** *Ansonia* sp. Usun Apau and *Ansonia minuta*, and **c** *Ansonia platysoma* and *Ansonia minuta*

evolutionary species concept (Wiley 1978), and the unified species concept (de Queiroz 2007).

Ansonia teneritas, sp. nov.

Holotype: NMBE 1057364, from a small stream on the upper reaches of the Sungei Julan on the northern side of the Usun Apau Plateau just above the falls of the Sungei Julan Valley, Usun Apau National Park, Sarawak, East Malaysia (Borneo). GPS N 03.03292°, E 114.66647°, 1002 m a.s.l, coll. A. Haas, Pui Yong Min, S.T. Hertwig & A. Jankowski, 28. August 2010. Adult male.

Paratypes: NMBE 1057318–1057327, ZRC 1.12525-12526, from the same locality as holotype, coll. A. Haas, Pui Yong Min, S.T. Hertwig & A. Jankowski, 27 and 28 August 2010; ZMH A11568, from Gunung Hose, Sarawak, East Malaysia (Borneo), GPS N 02.23526°, E 113.68693°, 1104 m asl., coll. A. Haas & Y.M. Pui, 25. August 2011.

Diagnosis

The new species is assigned to the genus *Ansonia* on the basis of a robustly supported phylogenetic hypothesis (Fig. 3) and the presence of the following morphological characters: body slender, subarticular tubercles indistinct, limbs long and slender, webbing membranous, parotid glands absent, and tympanum visible (Inger 1960, 1966).

Ansonia teneritas sp. nov. can be distinguished from all congeners by the following combination of characters: (1) SVL in males <22 mm, in females <25 mm; (2) snout clearly projecting in profile; (3) head depressed; (4) longitudinal interorbital ridges absent; (5) first finger short, not reaching base of tip of second finger when adpressed; (6) no sharp tarsal ridge; (7) skin flaps on posterior thigh near vent absent; (8) warts on temporal regions not enlarged; (9) coloration olive-green with a contrasting dark brown pattern on head and dorsum; (10) whitish pattern below the eye on upper lip; (11) white warts behind jaw joint, on shoulders and flanks; (12) iris bright red-orange with an irregular network of black reticulations.

Description of holotype

Habitus very slender (Fig. 5); body slightly wider than head; head depressed (Fig. 6); head shorter than it is wide; rostrum truncate in dorsal view; tip of snout rounded, longer than canthus, protruding in lateral view, clearly projecting beyond tip of mandible; rostral length shorter than eye diameter; nostril lateral, closer to snout tip than to eye; indistinct vertical ridge between tip of snout and centre of maxilla; canthus rounded; lores vertical and slightly concave; eye diameter greater than eye-nostril distance; interorbital distance slightly larger than the upper eyelid; tympanum round and distinct; eye diameter 3.48 times greater than tympanic diameter; distance from eye to tympanum 0.43 mm

Fingers slender; tips slightly expanded forming small, spatulate discs; first finger significantly shorter than second; nuptial pads with blackish brown spines on first finger; subarticular

Fig. 5 Coloration in life of a female *Ansonia teneritas* sp. nov. from the type locality on the Usun Apau plateau

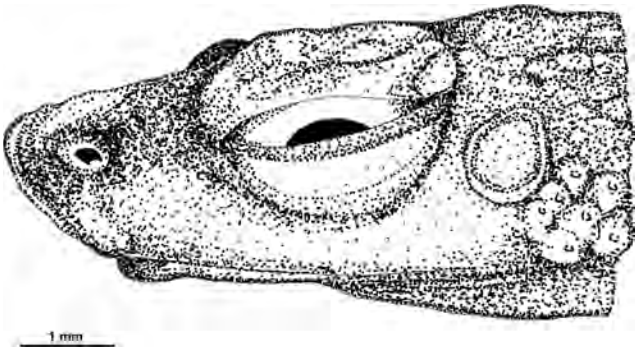

Fig. 6 Head of holotype of *Ansonia teneritas* sp. nov. in lateral view

tubercles weak and indistinct, exclusively below the proximal articulation of the first phalange, absent below distal phalanges; palm smooth with large, distinct rounded palmar tubercles; tips of all toes rounded; fifth toe slightly longer than third; membranous web reaching disc of first toe, disc on both sides of second toe, third toe with one and two-thirds phalanges free, fourth with three phalanges free, fifth toe with two phalanges free of web. Subarticular tubercles indistinct; two metatarsal tubercles, both oval and slightly raised; tarsal fold absent.

Skin of head, dorsum, flanks, and dorsal surfaces of limbs with scattered, isolated, flat, rounded warts of heterogeneous size, warts on temporal and dorsolateral regions not significantly larger (Figs. 5 and 7); warts on head, dorsum, flanks, and limbs often terminating in fine brown keratinous spines or caps; spines below the symphysis of mandibles absent, but one row of very small, spineless tubercles along the ventral face of the lower jaw; skin flaps on posterior thigh near vent absent.

Colour in preservative grayish brown with a contrasting dark gray pattern of bands and spots on head and dorsum beginning at the eyes and running to the forehead and shoulders, forming an x with a light spot in its center; irregular light spot below the eye on upper lip; limbs gray brown with contrasting lighter cross bands; venter pale yellowish white with dark mottling on throat, chest, and belly; small, whitish tubercles present on the pectoral region and the venter (Fig. 7).

Coloration in life olive-green with a contrasting dark brown pattern of irregular spots and bands, often forming an x-shape between the eyes, on forehead, and on shoulders; larger warts on dorsum yellow to orange, whitish warts ventrolaterally on flanks and from jaw joint to shoulder, an irregular whitish spot below the eye on the upper lip, limbs dark brown with yellow crossbars (Fig. 5); venter pale yellowish white with brown mottling, especially on throat and chest, and interspersed with whitish tubercles. Iris bright red-orange with an irregular network of black reticulations (Fig. 5).

Variation

The extent of the dark pattern on throat, chest, and venter varies from a few markings on the anterior part of the throat to intense mottling on throat, chest, and belly (Fig. 7). Some specimens possess up to three rows of very small, spineless tubercles along the ventral face of the lower jaws, while in other specimens, such

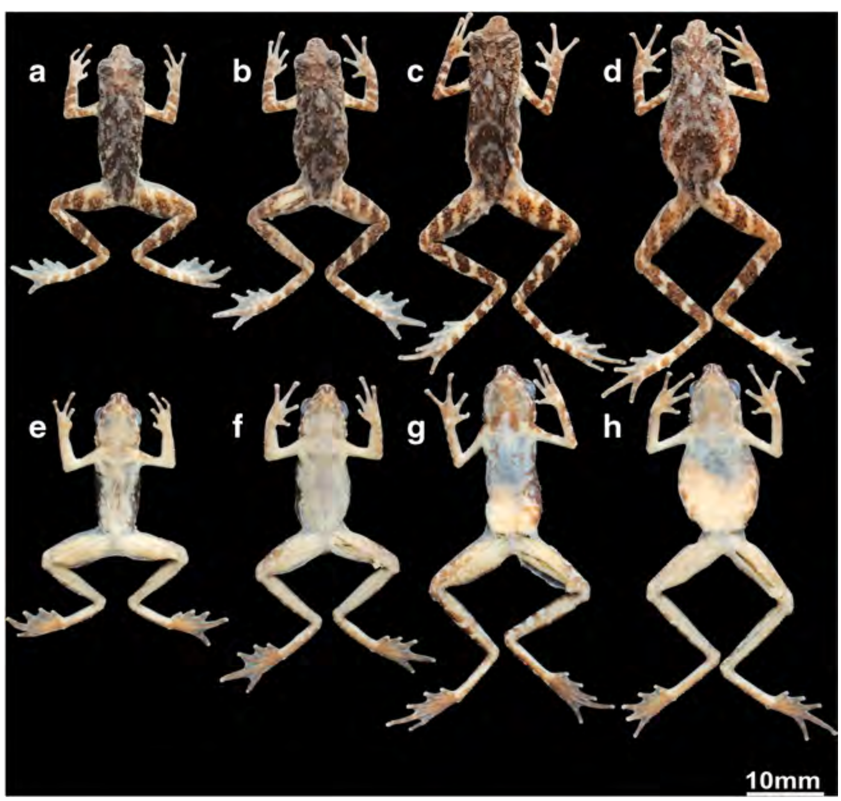

Fig. 7 Preserved type material of *Ansonia teneritas* sp.nov.: **a** dorsal view, **e** ventral view of male holotype (NMBE 1057364); **b** dorsal view, **f** ventral view of male paratype 1 (NMBE 1057326); **c** dorsal view, **g** ventral view of female paratype 2 (NMBE 1057321); **d** dorsal view, **h** ventral view of female paratype 3 (NMBE 1057327)

tubercles are lacking. There is no noticeable variation in the described characters between the specimens from Usun Apau National Park and the single female specimen from Gunung Hose, Sarawak.

Sexual dimorphism

Males are smaller (SVL 19.16–21.53 mm) than females (21.37–24.4 mm); males possess vocal sacs and nuptial pads with numerous blackish brown spines. Females have less extensive webbing: the first toe is fully webbed, the second toe is about one phalange free of web, the third toe about two, the fourth about three to three and a half, and the fifth about two to two and a half free of web.

Comparison

The following characters distinguish *Ansonia teneritas* sp. nov. from its other Bornean congeners: *Ansonia albomaculata* Inger 1960: tympanum obscured by skin; head not depressed; sharp tarsal ridge present; skin of body and limbs uniformly covered with small, round warts; an oblique light band from the posterior corner of the eye to axilla; no contrasting pattern on head, dorsum, or limbs (Inger 1966; Inger and Stuebing 2005). *Ansonia echinata*: black spines under mandible, large ventrolateral tubercles tipped with small black spines; spinose tubercles on top of snout with black spines, no contrasting pattern on head, dorsum, or limbs (Inger and Stuebing 2009). *Ansonia fuliginea*: larger body size (males 32–36 mm, females 38–44 mm); snout vertical in profile; head not depressed; no contrasting pattern on head, dorsum, or limbs, blackish on sides and underside (Inger 1960, 1966; Inger and Stuebing 2005). *Ansonia guibei* Inger 1966: snout vertical in profile; head not depressed; first finger long, reaching base of second when adpressed; fourth and second finger equal in length; membranous web reaching tips of third and fifth toes; skin dorsally and dorsolaterally with numerous round warts with clusters of dark spinules; oblique flap of skin present on each side of the vent; no contrasting pattern on head, dorsum, or limbs (Inger 1966; Inger and Stuebing 2005). *Ansonia hanitschi*: larger body size (males 20–28 mm, females 28–35 mm); males with a few spinose tubercles below symphysis of the mandibles; colour pattern on head, dorsum, and limbs obscure and less contrasting (Dring 1983; Inger 1966; Inger and Stuebing 2005); *Ansonia latidisca*: larger body size (males 35 mm, females 55 mm); head not depressed; snout almost vertical in profile; tips of the three outer fingers dilated into truncate discs, that of third as wide as tympanum; forelimb very long and slender; two rows of interorbital, conical tubercles; different colour pattern on head and back consisting of a bright green background with a distinct pattern of blackish brown spots and scattered orange or red warts (Inger 1966; Matsui et al. 2012 *Ansonia leptopus*: larger body size (males 35 mm, females 55 mm); head not depressed; first finger long, reaching disc of second; dorsolateral warts larger, males with rows of brown spines under mandible; no contrasting pattern on head, dorsum, or limbs (Inger 1966; Inger and Dring 1988; Inger and Stuebing 2005). *Ansonia longidigita*: larger body size (males 50 mm, females 65 mm); head not depressed; first finger long, reaching tip of disc of second; snout almost vertical in profile; a pair of longitudinal rows of tubercles in interorbital space; males with three to six rows of brown or black spines under mandible; no contrasting pattern on head or dorsum (Inger 1966; Inger and Dring 1988; Inger and Stuebing 2005; Matsui et al. 2010). *Ansonia minuta*: head not depressed; distinct tarsal ridge; nuptial pads absent; third and fifth toes webbed to disc in males; more warts on dorsal face of the head; warts on dorsum and flanks not spinose, dark pattern on dorsum less contrasting (Inger 1960, 1966; Inger and Stuebing 2005). *Ansonia platysoma*: warts on dorsum and flanks often without or with indistinct keratinous spines or caps; nuptial pads consisting of spines with a paler brown coloration; third toe usually webbed to disc in males; no white pattern on upper lip; no white warts on shoulders or flanks; colour pattern on head, dorsum, and limbs obscure and less contrasting (Inger 1960, 1966). *Ansonia spinulifer*: larger body size (males 30–40, females 40–45); head not depressed; first finger reaching tip of second when adpressed; dorsal and dorsolateral warts large, elevated and juxtaposed, tipped with one or three strong melanistic spines; males with spinose tubercles under mandibular symphysis and lower jaw; adults black on top with a light spot between the shoulders but without a contrasting pattern on head, dorsum, or limbs (Inger and Dring 1988; Inger and Stuebing 2005). *Ansonia torrentis* Dring 1983: larger body size (males 30–33 mm); head relatively wide in comparison to body; warts on dorsum without keratinous spines; males with spinose tubercles under mandibular symphysis and lower jaw (Dring 1983). *Ansonia vidua*: larger body size (females 30–34 mm); head not depressed; two low, curved longitudinal ridges present on the interorbital region; skin of back, flanks and upper surfaces of the limbs covered with numerous small, regular, flat, rounded warts resulting in a velvet-like appearance; coloration of head, body and limbs uniformly black-brown with no markings (Hertwig et al. 2014).

Etymology

The species name *teneritas*, Latin for ‘tenderness’, refers to the gracile body shape and small body size of this species in comparison to congeners. We suggest the English name ‘Gracile Slender Toad’.

Ecological notes

The type locality of *Ansonia teneritas* sp. nov. is a small tributary of the upper reaches of the Sungei Julan, just above the spectacular falls of the Sungei Julan Valley on the northern side of the Usun Apau plateau (Fig. 8). The Usun Apau plateau is part of the

Central Bornean plateau and forms the watershed between the Rejang and Baram river basins in central Sarawak (Hutchison 2005; Rousseau 1977). The Sungei Julan is a tributary of the Baram drainage system. It consist of several tablelands and smaller plateaus with an elevation of approximately 760–1000 m with sharply cut edges, precipitous cliffs, and deep marginal embayments (Hutchison 2005). The Usun Apau highlands were formed by extensive volcanic activity during the Upper Miocene to Quaternary, and the few mountains that stand over 300 m above the tablelands are relicts of former volcanoes (Hutchison 2005). As a result of their volcanic formation history the high tablelands of the central area are built up of dacite tuff and agglomerate, while the southern mountains are covered by basalt lava (Hutchison 2005; Campbell 1956). The area covers about 700 sq. km of forest and has been protected as the Usun Apau National Park since 2005.

The small stream at the type locality is presumably permanent and has a moderate gradient. The stream bed consists of alternating small shallow rocky pools (20 to 50 cm depth) and fast-running sections on bedrock (0.5 to 1.5 m in width, depending on the amount of precipitation) (Fig. 8). Below the type locality the stream flows to the Sungei Julan just below the upper tier of the Eastern Julan falls in the steep Sungei Julan Valley. The vegetation at the type locality is primary lower montane forest. On the surrounding hills the forest type changes to kerangas (Bornean heath) forest. All specimens of *Ansonia teneritas* sp. nov. were collected at night from rocks or low vegetation along the stream. Males call at night from saplings on the stream banks or on bedrock.

Adult frogs of the following species were detected in the immediate vicinity of the stream and the surrounding forest:

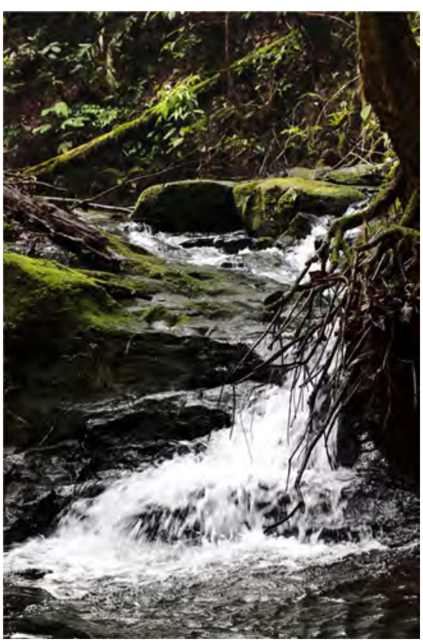

Fig. 8 Habitat at the type locality of *Ansonia teneritas* sp. nov. on the Usun Apau plateau

Ansonia longidigita Inger, 1960; *Hylarana picturata* (Boulenger 1920); *Leptobrachella mjobergi* Smith, 1925; *Limnonectes* cf. *kuhlii* (Tschudi, 1838); *Limnonectes ibanorum* (Inger 1964); *Meristogenys* sp., *Metaphrynella sundana* (Peters, 1867); *Microhyla petrigena* Inger and Frogner 1979; *Occidozyga laevis* (Günther 1858); *Philautus hosii* (Boulenger 1895); *Philautus macroscelis* (Boulenger 1896); *Philautus* cf. *mjobergi* Smith, 1925; *Philautus tectus* Dring, 1987; and *Staurois parvus* Inger and Haile, 1959.

The second locality from where *Ansonia teneritas* is known is the Gunung Hose, a relatively isolated, remote, and poorly explored mountain range in central Sarawak. The Gunung Hose range is composed of volcanic rock forming frequently steep cliff faces (Lee, 2002). The single specimen was found on low vegetation by a deeply entrenched small stream in a selectively logged secondary forest near a logging road. At the same locality adults of *Ansonia longidigita* Inger, 1960 and *Staurois guttatus* (Günther, 1858) were found; additionally, *Rhacophorus borneensis* Matsui, Shimada, and Sudin, 2013 was heard calling.

The larval stages and call of the new *Ansonia* remain undocumented.

Discussion

The phylogenetic hypothesis presented here suggest that *Ansonia teneritas* sp. nov. and *Ansonia torrentis* are members of a robustly supported monophyletic group consisting exclusively of species from Borneo: *Ansonia hanitschi*, *Ansonia minuta*, *Ansonia platysoma*, *Ansonia spinulifer*, *Ansonia vidua* and the undescribed taxa referred to as *Ansonia* sp. 3 and *Ansonia* sp. 4 (Fig. 3). Certain phylogenetic relationships within that clade, however, received moderate support values. Especially the sister group relationship of *Ansonia teneritas* to the group consisting of *Ansonia torrentis*, *Ansonia platysoma*, *Ansonia* sp.5 and *Ansonia vidua* was only moderately supported. We explain the limited support in BI and ML analyses of our sequence data in favor of the phylogenetic position of the new taxon as a result of analyzing only mitochondrial markers. In subsequent studies the closest relatives of *Ansonia teneritas* should be confirmed, therefore, by using additional mitochondrial data and, in particular, fast evolving nuclear markers. Nevertheless we present robust genetic evidence that *Ansonia torrentis* is not a close relative of *Ansonia longidigita, Ansonia leptopus*, *Ansonia latifii* or *Ansonia* sp. 5 (Matsui et al. 2010, Fig. 3). In contrast, the sample AB435296, labeled as *Ansonia torrentis* by Matsui et al. (2010), clustered with *Ansonia longidigita* in previous phylogenetic analyses (Hertwig et al. 2014; Matsui et al. 2010) and also in the tree presented here (Fig. 3). We assume, therefore, that sample AB435296 has been erroneously assigned to *Ansonia torrentis* and must be regarded instead as a sample belonging to *Ansonia longidigita*.

Within the above mentioned group *Ansonia hanitschi, Ansonia minuta*, *Ansonia platysoma*, *Ansonia teneritas* and *Ansonia torrentis* are genetically clearly distinct but superficially similar in size, shape, and colour pattern, and therefore represent so called cryptic species. The results of our integrative approach support the taxonomic status of *Ansonia teneritas* as a distinct species on the basis of a unique combination of genetic, morphometric, and morphological traits. In scientific descriptions of anurans a considerable number of body measurements are commonly documented in order to distinguish between related species. In recent taxonomic studies on *Ansonia* up to 30 measurements were used (e.g., Boulenger 1880, 1897; Gascon et al. 1996; Heyer 1984; Matsui 1984; Okada 1931). Beyond the undisputed descriptive value of these measurements their utility in the unequivocal diagnosis and determination of cryptic species in *Ansonia* has not been demonstrated. This study therefore made use of the approach proposed by Baur and Leuenberger (2011) to test the discriminatory power of the morphometric measurements traditionally used. The combination of shape PCA 1 and 2 failed to separate the species from each other due to a significant overlap in morphometric ratios in both sexes, but particularly in males. Nevertheless, we identified a set of morphometric ratios that permitted the reliable discrimination of *Ansonia minuta*, *Ansonia platysoma* and *Ansonia teneritas* from each other on the basis of just six measurements (SVL, HD, IN, NML, FOT, THL, Fig. 4). The contribution of HD to the separation of *Ansonia platysoma* from the remaining species is in accordance with the original description of this species by Inger (1966), who mentioned a flattened habitus and depressed head.

The four morphometric measurements AGD, BW, tympanum-eye distance (TED), and tympanum-mouth length (TML) exhibited exceptionally high variance and were therefore excluded from the final statistical analysis (see also Hayek et al. 2001). The observed variance can be explained as the result of a combination of natural variability, preservation artifacts, and/or measurement errors. TED and TML may additionally be influenced by accuracy issues, as both are especially short distances with one point of reference formed by the circular tympanum. AGD and BW are particularly likely to be influenced by preservation artifacts, as well as being subject to high natural variability caused by reproductive cycle and nutritional status. Morphometric measurements that are defined by osseous landmarks should therefore be preferred over soft body measurements in species descriptions and morphometric analyses (Hayek et al. 2001; Lee 1982). Measurement errors should only play a minor role in our data because we used carefully adjusted photos for each morphometric distance and a calibrated, industry-standard system which offers much greater accuracy than the use of hand-held calipers.

Ansonia spinulifer, which represents the sister taxon to the closely related species of the Bornean clade described above, is a relatively large species widespread in hilly lowland to lower montane forests in Sarawak, Brunei, and Sabah. In contrast, *Ansonia hanitschi*, *Ansonia minuta*, *Ansonia platysoma*, *Ansonia teneritas* and *Ansonia vidua* are only known from a few isolated spots of suitable habitat at varying elevations and, except for the two syntopic species *Ansonia hanitschi* and *Ansonia torrentis* on Gunung Mulu, exhibit allopatric or parapatric distribution patterns the two syntopic species *Ansonia hanitschi* and *Ansonia torrentis* on Gunung Mulu (Fig. 1). We regard the allopatric/parapatric species as mutual ecological replacements that have resulted from a non-adaptive radiation on Borneo (Rundell and Price 2009). We have to stress, however, that the phylogenetic relationships and taxonomic status of several isolated populations within the ranges of the described species and the undescribed taxa have yet to be confirmed. We expect the biogeography and evolutionary history of this monophyletic group of Bornean *Ansonia* to be more complex than current knowledge may suggest. The pronounced difference in body size between the syntopic species *Ansonia hanitschi* and *Ansonia torrentis* (Dring, 1983) can be explained as the result of character displacement. In the case of *Ansonia vidua*, which is morphologically distinct from the other species of this clade in its larger body size, skin structure and colour pattern, a lack of detailed ecological data prevents a reliable interpretation of the evolutionary history of this enigmatic taxon.

Ansonia teneritas is currently only known from elevations above 1100 m asl in the Gunung Hose range and the Usun Apau plateau in central Sarawak and must be regarded as being endemic to central Sarawak and having a disjunct, montane distribution (Fig. 1). The only known localities in which *Ansonia teneritas* have been found so far are small, clear streams in montane forest (Fig. 8) in the two isolated mountain ranges Usun Apau and Gunung Hose (Fig. 1). According to data available on other species of this genus, the adaptability of *Ansonia teneritas* to disturbed habitats and its ability to survive episodes of intense logging and resulting high siltation in the streams used for breeding is probably limited. However, the single specimen from Gunung Hose found in a disturbed forest creates the hope that this species may be able to adapt to secondary habitats of acceptable quality. Further studies into the ecology of *Ansonia teneritas* and its close relatives are urgently needed in order to close the gap in our knowledge of the habitat preferences, abundance, adaptability, and distribution of this species. The high altitude and extremely rugged topography of Usun Apau and Gunung Hose have prevented logging activity at higher elevations in the past. However, strict protection of montane forests to avoid degradation or loss—particularly of the water bodies suitable for reproduction—will be crucial to the long-term survival of these endemic toads of central Sarawak. Future conservation efforts directed toward *Ansonia teneritas* and the related montane *Ansonia* will need to focus on enduringly preserving the highly diverse fauna and flora of montane forests in the mountain ranges of Sarawak and Sabah. So far, only the Usun Apau plateau is protected by law as a national park.

Acknowledgments The Economic Planning Unit of the Prime Minister's Department, Malaysia, especially Munirah Abd. Manan, helped by issuing permission to conduct research in Malaysia. We thank the Sarawak Forest Department and Sarawak Forestry Corporation, in particular Nur Afiza binti Umar, Dayang Nuriza binti Abang Abdillah, Mohamad bin Kohdi, Engkamat anak Lading, Oswald Braken Tisen, Datu Haji Ali Yusop, and Mohd. Shabudin Sabki, for providing advice and issuing permits. Our Usun Apau expedition benefited significantly from Willie Kajan's knowledge and organization. Hannes Baur is thanked for his help with morphometric analyses, while Beatrice Blöchlinger and Chris Sherry performed technical tasks with admirable skills. We are particularly grateful to the Burgergemeinde Bern and Leder Fuchs, Göppingen, for their generous support of our fieldwork. For permission to examine material under their care, we thank Colin McCarthy (BMNH), Alan Resetar and Robert Inger (FMNH), Margarita Naming (SBC), Charles Leh Moi Ung (SM), David Edwards and Helen Pang (UBD), and Peter Kee Lin Ng and Kelvin Kok Peng Lim (ZRC). PYM and ID were supported by NRGS/1087/2013(01). Finally, we thank Lucy Cathrow who revised the language of this manuscript and two anonymous reviewers whose helpful comments contributed significantly to the improvement of this publication.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Appendix

Table 3 Locality data, collection numbers, and GenBank accession numbers of the specimens used in the phylogenetic analysis of this study

Species	Locality	Voucher	Genbank	Author
Ansonia albomaculata	East Malaysia, Sarawak, Lanjak Entimau	KUHE 17377	AB435303	Matsui et al. (2010)
Ansonia albomaculata	East Malaysia, Sarawak, Lanjak Entimau	KUHE 17499	AB435304	Matsui et al. (2010)
Ansonia albomaculata	East Malaysia, Sarawak, Lanjak Entimau	KUHE 17503	AB435305	Matsui et al. (2010)
Ansonia endauensis	West Malaysia, Johor, Endau-Rompin	LSUHC 7726	AB435257	Matsui et al. (2010)
Ansonia fuliginea	East Malaysia, Sabah, Kinabalu, Pakka	BOR 22770	AB331709	Matsui et al. (2010)
Ansonia fuliginea	East Malaysia, Sabah, Kinabalu, Pakka	KUHE 17537	AB435308	Matsui et al. (2010)
Ansonia guibei	East Malaysia, Sabah, Kinabalu, Mesilau	BOR 22928	AB435306	Matsui et al. (2010)
Ansonia guibei	East Malaysia, Sabah, Kinabalu, Mesilau	KUHE L06B054	AB435307	Matsui et al. (2010)
Ansonia hanitschi	East Malaysia, Sabah, Gunung Kinabalu NP	ZMH A09248	KX259286	This study
Ansonia hanitschi	East Malaysia, Sabah, Gunung Kinabalu NP	ZMH A12560	KX259283	This study
Ansonia hanitschi	East Malaysia, Sabah, Kinabalu Park	ZMH A12559	KX259284	This study
Ansonia hanitschi	East Malaysia, Sabah, Kinabalu, Silau Silau	BOR 22640	AB331710	Matsui et al. (2010)
Ansonia hanitschi	East Malaysia, Sabah, Kinabalu, Silau Silau	BOR 22641	AB435277	Matsui et al. (2010)
Ansonia hanitschi	East Malaysia, Sabah, Kinabalu, Silau Silau	BOR 22642	AB435278	Matsui et al. (2010)
Ansonia inthanon	Thailand, Doi Inthanon	KUHE 19050	AB435253	Matsui et al. (2010)
Ansonia jeetskumarani	West Malaysia, Pahang, Fraser's Hill	LSUHC 8049	AB435265	Matsui et al. (2010)
Ansonia jeetskumarani	West Malaysia, Pahang, Fraser's Hill	LSUHC 8050	AB435266	Matsui et al. (2010)
Ansonia kraensis	Thailand, Ranong	KUHE 23517	AB435250	Matsui et al. (2010)
Ansonia kraensis	Thailand, Ranong	KUHE 35814	AB435251	Matsui et al. (2010)
Ansonia kraensis	Thailand, Ranong	KUHE 35817	AB435252	Matsui et al. (2010)
Ansonia latiffi	West Malaysia, Pahang, Sg. Lembing	LSUHC 4991	AB435299	Matsui et al. (2010)
Ansonia latiffi	West Malaysia, Pahang, Sg. Lembing	LSUHC 4992	AB435300	Matsui et al. (2010)
Ansonia latirostra	West Malaysia, Pahang, Sg. Lembing	LSUHC 4923	AB435260	Matsui et al. (2010)
Ansonia latirostra	West Malaysia, Pahang, Sg. Lembing	LSUHC 4924	AB435261	Matsui et al. (2010)
Ansonia leptopus	East Malaysia, Sabah, Tawau Hills NP	UNIMAS X8961	KX259316	This study
Ansonia leptopus	East Malaysia, Sabah, Tawau Hills NP	BOR 22139	AB435297	Matsui et al. (2010)
Ansonia leptopus	East Malaysia, Sarawak, Gunung Gading NP	KUHE 17109	AB435298	Matsui et al. (2010)
Ansonia leptopus	East Malaysia, Sarawak, Gunung Gading NP	NMBE 1059720	KX259312	This study
Ansonia leptopus	East Malaysia, Sarawak, Gunung Mulu NP	UNIMAS 8968	KX259289	This study
Ansonia leptopus	East Malaysia, Sarawak, Kampung Sebako	ZMH A12558	KX259291	This study
Ansonia leptopus	East Malaysia, Sarawak, Kampung Sebako	ZMH A12557	KX259290	This study
Ansonia longidigita	East Malaysia, Sabah, Bundu Tuhan	KUHE L04B133	AB435294	Matsui et al. (2010)
Ansonia longidigita	East Malaysia, Sabah, Crocker Range NP	ZMH A09370	KX259285	This study
Ansonia longidigita	East Malaysia, Sabah, Crocker, Mahua	BOR 12463	AB331711	Matsui et al. (2010)

Table 3 (continued)

Species	Locality	Voucher	Genbank	Author
Ansonia longidigita	East Malaysia, Sabah, Crocker, Ulu Kimanis	BOR 12862	AB435293	Matsui et al. (2010)
Ansonia longidigita	East Malaysia, Sabah, Sungai Kemantis trail	UNIMAS X9043	KX259317	This study
Ansonia longidigita	East Malaysia, Sarawak, Bario	KUHE-12405	AB435295	Matsui et al. (2010)
Ansonia longidigita	East Malaysia, Sarawak, Gunung Mulu NP	NMBE 1056284	KX259280	This study
Ansonia longidigita	East Malaysia, Sarawak, Pulong Tau NP	NMBE 1061668	KX259308	This study
Ansonia longidigita	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057282	KX259294	This study
Ansonia longidigita (Ansonia torrentis)	East Malaysia, Sarawak, Gn. Mulu NP	ZRC 1.11918	AB435296	Matsui et al. (2010)
Ansonia malayana	West Malaysia, Larut	KUHE 15467	AB331712	Matsui et al. (2010)
Ansonia malayana	West Malaysia, Larut	KUHE 15472	AB435264	Matsui et al. (2010)
Ansonia mcgregori	Philippines, Mindanao	ACD3600	AB435316	Matsui et al. (2010)
Ansonia mcgregori	Philippines, Mindanao	ACD3601	AB435317	Matsui et al. (2010)
Ansonia minuta	East Malaysia, Sarawak, Mount Penrissen	NMBE 1057517	KX259306	This study
Ansonia minuta	East Malaysia, Sarawak, Mount Penrissen	NMBE 1057518	KX259307	This study
Ansonia minuta	East Malaysia, Sarawak, Mount Penrissen	NMBE 1061561	KX259309	This study
Ansonia minuta	East Malaysia, Sarawak, Kubah NP	NMBE 1059724	KX259310	This study
Ansonia minuta	East Malaysia, Sarawak, Kubah NP	NMBE 1059725	KX259311	This study
Ansonia minuta	East Malaysia, Sarawak, Kubah NP	ZMH A12555	KX259293	This study
Ansonia minuta	East Malaysia, Sarawak, Kubah NP	UNIMAS 8862	KX259288	This study
Ansonia minuta	East Malaysia, Sarawak, Kuching	KUHE 12058	AB435281	Matsui et al. (2010)
Ansonia minuta	East Malaysia, Sarawak, Kuching	KUHE 17233	AB435282	Matsui et al. (2010)
Ansonia minuta	East Malaysia, Sarawak, Kuching	KUHE 17274	AB435283	Matsui et al. (2010)
Ansonia minuta	East Malaysia, Sarawak, Santubong NP	NMBE 1059717	KX259313	This study
Ansonia minuta	East Malaysia, Sarawak, Santubong NP	NMBE 1059718	KX259314	This study
Ansonia minuta	East Malaysia, Sarawak, Santubong NP	NMBE 1059719	KX259315	This study
Ansonia muelleri	Philippines, Mindanao	ACD3543	AB435309	Matsui et al. (2010)
Ansonia muelleri	Philippines, Mindanao, Davao City	RMB 639	AB435310	Matsui et al. (2010)
Ansonia muelleri	Philippines, Mindanao, Davao City	RMB 642	AB435311	Matsui et al. (2010)
Ansonia muelleri	Philippines, Mindanao, Davao, Mt. Apo	ACD 1617	AB435312	Matsui et al. (2010)
Ansonia muelleri	Philippines, Mindanao, Davao, Mt. Apo	ACD 1640	AB435313	Matsui et al. (2010)
Ansonia muelleri	Philippines, Mindanao, Mt. Hamigitan	ACD 2631	AB435314	Matsui et al. (2010)
Ansonia muelleri	Philippines, Mindanao, Mt. Hamigitan	ACD 2702	AB435315	Matsui et al. (2010)
Ansonia penangensis	West Malaysia, Penang Is.	KUHE UNL	AB435262	Matsui et al. (2010)
Ansonia penangensis	West Malaysia, Penang Is.	KUHE UNL	AB435263	Matsui et al. (2010)
Ansonia platysoma	East Malaysia, Sabah, Crocker Range	BOR 12499	AB435267	Matsui et al. (2010)
Ansonia platysoma	East Malaysia, Sabah, Crocker Range	BOR 12500	AB435268	Matsui et al. (2010)
Ansonia platysoma	East Malaysia, Sabah, Gunung Kinabalu NP	ZMH A12556	KX259287	This study
Ansonia platysoma	East Malaysia, Sabah, Kinabalu, Bundu Tuhan	KUHE L04B131	AB435270	Matsui et al. (2010)
Ansonia platysoma	East Malaysia, Sabah, Kinabalu, Bundu Tuhan	KUHE L04B132	AB435271	Matsui et al. (2010)
Ansonia platysoma	East Malaysia, Sabah, Kinabalu, Poring	ZRC 1.11917	AB435272	Matsui et al. (2010)
Ansonia platysoma	East Malaysia, Sabah, Kinabalu, Sayap	BOR 23347	AB435269	Matsui et al. (2010)
Ansonia siamensis	Thailand, Khaochong	KUHE 23438	AB435255	Matsui et al. (2010)
Ansonia siamensis	Thailand, Khaochong	KUHE 23515	AB435256	Matsui et al. (2010)
Ansonia sp. 1	Thailand, Kanchanaburi, Pilok	KUHE 35066	AB435249	Matsui et al. (2010)
Ansonia sp. 2	Thailand, Pukhet	KUHE 38071	AB435254	Matsui et al. (2010)
Ansonia sp. 3	East Malaysia, Sarawak, Bario	KUHE 12380	AB435273	Matsui et al. (2010)
Ansonia sp. 4	East Malaysia, Sabah, Crocker, Ulu Kimanis	BOR 08475	AB435280	Matsui et al. (2010)
Ansonia sp. 4	East Malaysia, Sarawak, Bario	KUHE 12433	AB435274	Matsui et al. (2010)
Ansonia sp. 4	East Malaysia, Sabah, Crocker, Ulu Kimanis	BOR 08424	AB435279	Matsui et al. (2010)
Ansonia sp. 5	East Malaysia, Sarawak, Bario	KUHE 12434	AB435275	Matsui et al. (2010)
Ansonia sp. 5	East Malaysia, Sarawak, Lanjak Entimau	KUHE 17381	AB435301	Matsui et al. (2010)
Ansonia sp. 5	East Malaysia, Sarawak, Lanjak Entimau	KUHE 17486	AB435302	Matsui et al. (2010)
Ansonia sp. 6	East Malaysia, Sarawak, Bario	KUHE 12448	AB435276	Matsui et al. (2010)

Table 3 (continued)

Species	Locality	Voucher	Genbank	Author
Ansonia spinulifer	East Malaysia, Sabah, Crocker, Ulu Kimanis	KUHE L04B138	AB435286	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sabah, Crocker, Ulu Kimanis	KUHE L04B139	AB435287	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sabah, Crocker, Ulu Kimanis	BOR 08433	AB435288	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sabah, Maliau	KUHE L05B005	AB435285	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sabah, Tawau	BOR 09247	AB435284	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sarawak, Gading	KUHE 17182	AB435292	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sarawak, Kuching	KUHE 12065	AB435289	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sarawak, Sadong River	LSUHC 4046	AB435290	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sarawak, Sadong River	LSUHC 4047	AB435291	Matsui et al. (2010)
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Gunung Hose	ZMH A11568	KX259292	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	ZRC 1.12526	KX259296	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057325	KX259302	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057320	KX259297	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057364	KX259305	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	ZRC 1.12525	KX259295	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057326	KX259303	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057323	KX259300	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057322	KX259299	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057327	KX259304	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057321	KX259298	This study
Ansonia teneritas sp.nov.	East Malaysia, Sarawak, Usun Apau NP	NMBE 1057324	KX259301	This study
Ansonia tiomanica	West Malaysia, Pahang, Tioman Is.	LSUHC 2616	AB435258	Matsui et al. (2010)
Ansonia tiomanica	West Malaysia, Pahang, Tioman Is.	LSUHC 4443	AB435259	Matsui et al. (2010)
Ansonia torrentis	East Malaysia, Sarawak, Gunung Mulu NP	NMBE 1056297	KX259281	This study
Ansonia torrentis	East Malaysia, Sarawak, Gunung Mulu NP	NMBE 1056298	KX259282	This study
Ansonia vidua	East Malaysia, Sarawak, Pulong Tau NP	NMBE 1066153	KJ488547	This study
Ansonia vidua	East Malaysia, Sarawak, Pulong Tau NP	NMBE 1061645	KJ488546	This study
Duttaphrynus melanostictus (=Bufo melanostictus)	West Malaysia, Penang Is.	KUHE 39029	AB435318	Matsui et al. (2010)
Leptophryne borbonica	East Malaysia, Sabah, Crocker, Ulu Kimanis	BOR 08127	AB331716	Matsui et al. (2010)
Pelophylyne signata	West Malaysia, Genting	KUHE 35585	AB331720	Matsui et al. (2010)
Pelostibes hosii	East Malaysia, Sabah, Tawau	BOR 22088	AB331717	Matsui et al. (2010)
Sabahphrynus maculatus (=Pedostibes maculatus)	East Malaysia, Sabah, Crocker, Ulu Kimanis	BOR 08425	AB331718	Matsui et al. (2010)
Sabahphrynus maculatus (=Ansonia anotis)	East Malaysia, Sabah, Kinabalu	SP 26033	AB331708	Matsui et al. (2010)

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